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Effect of iron status on the intestinal absorption of aluminum: A reappraisal

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Effect of iron status on the intestinal absorption of aluminum: A reappraisal. Clinical and experimental studies have shown that serum aluminum (Al) is bound to transferrin and that cellular uptake of Al appears to be mediated by transferrin receptors. Based on these findings it is widely believed that intestinal Al absorption occurs via iron-specific, transferrin-dependent pathways and that iron (Fe) deficiency increases the intestinal absorption of Al. However, since no transferrin receptors are expressed on the absorptive surface of small intestinal epithelial cells this notion is doubtful. To further clarify the issue the present study investigated the effect of marked alterations of body Fe stores on the intestinal absorption of Al using three different rat models. (I) Serum Al concentrations and urinary excretion rates of Al were measured in iron-overloaded (Fe+) or iron-deficient (Fe−) rats with either normal (C) or impaired (5/6 nephrectomy) renal function (Nx) employing oral Al loads in single dose studies. (II) Tissue Al accumulation as well as serum and urine Al were determined in respective experimental groups exposed to a prolonged (41 days) dietary Al load. (III) To assess the effect of Fe status on the intestinal absorption of Al directly at the organ level perfusions of *in situ* rat gut preparations were performed. In the single dose studies administration of Al resulted in similar urinary excretion rates of Al in intact kidney groups (C+Fe−, 229 ± 85 nmol/5 days; C+Fe+, 240 ± 59 nmol/5 days) despite marked differences in liver Fe (C+Fe−, 1.34 ± 0.16 vs. C+Fe+, 55.69 ± 13.20 μmol/g) and duodenal mucosal Fe (C+Fe−, 0.68 ± 0.11 vs. C+Fe+, 3.17 ± 0.82 μmol/g). In addition, mucosal Al concentration 24 hours after the load was not affected by the Fe status (C+Fe−, 37 ± 16 nmol/g, C+Fe+, 56 ± 19 nmol/g). Regardless of the Fe status post-load Al excretion was enhanced in Nx rats (Nx+Fe−, 533 ± 234 nmol/five days, Nx+Fe+, 536 ± 201 nmol/five days). Irrespective of Fe status a prolonged dietary Al load resulted in a similar increase in tissue Al concentration (nmol/g) in liver (baseline, 159 ± 22; C+Fe−, 276 ± 125; C+Fe+, 251 ± 71; Nx+Fe−, 330 ± 119; Nx+Fe+, 437 ± 67) and in bone (baseline, 219 ± 119; C+Fe−, 433 ± 174, C+Fe+, 485 ± 141; Nx+Fe−, 504 ± 185; Nx+Fe+, 548 ± 215). The increase in spleen Al was significantly larger in Fe-overloaded rats (baseline, 194 ± 20; C+Fe+, 511 ± 129 vs. C+Fe−, 308 ± 62, $P < 0.05$; Nx+Fe+, 514 ± 67 vs. Nx+Fe−, 389 ± 119, $P < 0.05$). Brain Al tended to rise in Nx rats only (baseline, 96 ± 33; Nx+Fe+, 174 ± 100, Nx+Fe−, 156 ± 78, P = NS). Analogous results were obtained in *in situ* intestinal perfusion studies: Fe deficiency and Fe overload both did not affect the time-dependent increase in serum Al in either systemic or portal vein blood. When paracellular intestinal permeability was assessed mannitol absorption was significantly higher in uremic animals as compared to controls. Pharmacological blockade (2 mM kinetin) of the paracellular permeability substantially reduced the time-dependent increase in serum Al in uremic rats but had little effect in control animals, suggesting that even the excess absorption of Al observed in uremia occurs via a paracellular rather than an iron-specific pathway. In conclusion, the findings of the present study provide several lines of evidence against the commonly accepted view that the intestinal absorption of Al occurs via iron-specific pathways. Most likely, this is related to the fact, that neither the absorption of Fe nor the absorption of Al are mediated via transferrin receptors. In addition, the enhanced intestinal absorption of Al observed in uremic rats does also not occur via iron-specific pathways, but seems to be due to increased paracellular permeability of the intestine.

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Aluminum intoxication in patients with chronic renal failure is commonly related either to the use of aluminum-contaminated dialysis fluids or to the ingestion of aluminum-containing phosphate-binding drugs [1]. Avoidance of aluminum-contaminated water in the dialysis process as well as preferred prescription of aluminum-free phosphate binders have greatly diminished the prevalence of aluminum-related diseases. Bone biopsy-based studies have shown that the prevalence of aluminum-related bone disease may now range from a recent 27% [2, 3] to values below 3% [4–6], and thus to be considerably lower than reported in earlier studies [7, 8]. However, some degree of aluminum accumulation resulting in subtle or overt toxicity may persist in a certain number of dialysis patients. This finding is largely related to intestinal absorption from low doses of aluminum-containing phosphate binders and from other dietary sources [3, 9–11]. In addition, several episodes of acute and, in some instances, fatal aluminum intoxication have alerted the medical community and have drawn attention to the fact that aluminum toxicity will still have to be considered in the future [12, 13]. In this regard precise delineation of conditions that may predispose to enhanced accumulation or toxicity of aluminum will further facilitate monitoring and treatment of patients at risk. With respect to intestinal absorption, several factors such as citrate and other complexing agents, young age and the uremic state itself have been shown to increase the bioavailabilty and the intestinal absorption rate of aluminum [9, 11, 14–17].

In particular, iron deficiency has been suggested to promote the intestinal absorption of aluminum [18], and this latter finding would be of general importance in view of the increasing prevalence of absolute or functional iron deficiency related to the widespread use of human recombinant erythropoietin in patients with chronic renal failure [19, 20]. Since serum aluminum is bound to transferrin and since cellular uptake of aluminum is mediated by transferrin receptors [21–23], it was felt that iron deficiency might increase aluminum absorption via increased expression of transferrin receptors [18, 24]. However, because no transferrin

receptors are expressed on the absorptive surface of small intestinal epithelial cells, this notion is doubtful [25–27]. Moreover, the data base regarding the interrelationship between iron deficiency and enhanced absorption of aluminum is scarce and little information is available as to what degree other factors such as dietary complexing constituents or the uremic status may affect the increase in aluminum absorption in iron deficiency [11, 18, 28–33]. Therefore, the current study was undertaken to investigate whether marked alterations of body iron stores would indeed consistently affect the intestinal absorption of aluminum and, if so, whether the presence of the uremic milieu might modulate this effect. Three different experimental models were used to address these questions: (a) single dose studies with subsequent monitoring of serum concentrations and urinary excretion rates of aluminum; (b) extended exposure to a large dietary aluminum load with subsequent measurement of the tissue burden of aluminum in various organs; and (c) *in situ* intestinal perfusion studies. The results strongly argue against the notion that iron deficiency increases the fractional absorption of aluminum and they do not suggest that iron overload may protect from intestinal aluminum absorption.

Methods

Manipulation of renal function and of iron status

Male outbred Sprague-Dawley rats (Han:SPRD, Department of Laboratory Animal Research, RWTH) weighing 180 to 220 g were kept on a standard laboratory diet containing 200 mg/kg (3.58 mmol/kg) iron (Fe), 0.80% calcium, and 0.75% phosphorus supplemented with 400 U/kg vitamin D_3 (Eggersmann, Rinteln, Germany). To compensate for genetic differences all litter mates were distributed equally among the test groups prior to the experiments as described previously [34]. Renal failure groups were rendered uremic by a two-stage 5/6 nephrectomy (Nx) [15]. Two weeks after the second operation rats matched for weight and residual renal function were allocated to 3 groups of 10 rats each: Fe deficiency (Nx+Fe−), Fe overload (Nx+Fe+), and unchanged Fe status (Nx). In parallel, rats with intact kidneys were split into respective treatment groups (C+Fe−, C+Fe+, and C). Subsequently, all animals had free access to distilled water, but were pair-fed with specific diets: Fe deficiency was produced by feeding a low Fe diet (6 mg [107 μmol] Fe per kg) (C 1038; Altromin, Lage, Germany) for four weeks together with removal of 12 ml blood. Two ml blood were drawn by tail vein puncture on days 8, 12, 15, 19, 23, and 26. Fe loading was accomplished by intramuscular injection of 1 ml Fe dextran (Ferrum Hausmann, Asta Pharma, Frankfurt, Germany; 50 mg [895 μmol] Fe/ml) on days 8, 13, 16, 19, and 24 as well as feeding the same diet supplemented with Fe [900 mg (16.1 mmol)/kg]. Uremic rats and controls with normal Fe status received a basic feed containing 180 mg (3.2 mmol) Fe per kg but otherwise identical with the low Fe diet (C 1000, Altromin). The efficacy of Fe removal was ascertained by weekly determinations of hemoglobin, red blood cell counts and erythrocyte indices. Subsequently, rats were studied either by oral or by intravenous aluminum (Al) loads or by the intestinal perfusion technique.

For the long-term exposure study induction of Fe deficiency or Fe overload followed the same protocol. During dietary exposure to Al Fe overload and deficiency were maintained by weekly intramuscular injections of 50 mg Fe and weekly removal of 2 ml blood, respectively.

Upon termination of each experimental series nonheme Fe was measured in liver tissue and duodenal mucosa of all animals: Rats were anesthetized with ether and underwent median laparotomy. Following cannulation of the abdominal aorta ligatures were placed at the subdiaphragmatic portion and proximal to the aortic bifurcation. A cold perfusion (4°C) with 0.9% NaCl was started at a flow rate of 10 ml/min and continued until mesenteric vessels had become colorless and until the transected hepatic vein showed a clear effluent. Thereafter, the liver and duodenum were excised. The duodenum was opened, rinsed, and the mucosa was then stripped from the serosa with a glass slide and the content of nonheme Fe was determined in both tissues.

Single dose oral or intravenous aluminum loads

In all six study groups intestinal absorption of Al following a single oral load was studied according to a well established protocol [15, 17, 35]. Seventy-two hours prior to Al ingestion rats were placed in individual metabolic cages. Forty hours later, food was withdrawn but access to deionized drinking water was permitted until one hour prior to ingestion of Al. Each rat received a single oral load of 407 μmol (11 mg) Al administered as $AlCl_3$ in 1 ml of deionized water by gastric lavage. For baseline urinary excretion rates of Al 24-hour urine collections were obtained twice, and following the Al load urine was collected for five days. Water and food were returned two hours and 16 hours after administration of Al, respectively. Post-load all rats were placed on the Fe-deficient C 1038 diet to rule out pre-analytical errors due to differences in diets. Fecal Al contamination of urine was prevented by frequent and scrupulous cleaning of the metabolic cages with deionized water (Al content <70 nmol/liter) and by fasting the rats prior to and after the Al load.

In a separate series of experiments animals were dosed orally with Al and blood was drawn at hours 0, 1, 5 and 24. An additional series of experiments addressed the kinetics of mucosal Al in the duodenum following an oral Al load in Fe-overloaded or Fe-deficient animals. At hours 0, 5, and 24 duodena were prepared as described above. Subsequently, to remove surface bound Al preparations were washed three times with a citrate buffer (5 mM, pH 7.4) according to a previously described method [36]. Tissue Al was determined in mucosal pellets after the third wash.

To evaluate differences in tissue binding and renal handling of Al related to changes in Fe status and/or renal function, a further set of six experimental groups was studied employing intravenous administration of Al. A slow i.v. injection of 370 nmol (10 μg) Al ($AlCl_3$) in 0.5 ml deionized water was delivered over 10 minutes. Thereafter, the respective groups were handled as outlined above and recovery of Al in urine was monitored for five days.

In the final part of these experiments rats with normal renal function and unaltered Fe status were studied with single oral loads of 204 nmol (5.5 mg) Al administered as $AlCl_3$ concurrent with 204 nmol (11.4 mg) Fe ($FeCl_3$) or an equimolar amount of NaCl adjusted to the same pH. Again, recovery of Al was monitored in urine for a total of five days.

Long-term exposure to a dietary aluminum load

To further determine the effect of Fe status on the absorption and compartmentalization of Al uremic rats were exposed to a prolonged dietary Al load. Nx rats were either Fe-depleted or

Fe-overloaded as described. Thereafter, these animals and respective uremic controls were pair-fed an Al-supplemented [8 g (296 mmol)/kg Al, $Al(OH)_3$] low Fe [6 mg (107 μmol)/kg] diet together with drinking water containing 1250 mg/liter (46.3 mmol/liter) Al (Al lactate) and 100 g/liter (292 mM) sucrose. To avoid changes in Al bioavailability due to differences in the Fe content of the diet all groups were fed the same low Fe diet. However, alteration of Fe status was maintained by Fe injections or serial bleedings, and at the end of the experiment Fe status was confirmed by measurement of nonheme Fe in liver tissue and duodenal mucosa. After 41 days of exposure to this large dietary Al load rats were transferred to individual metabolic cages. Food was withdrawn to prevent fecal contamination of urine and distilled water (Al content < 70 nmol/liter) was supplied as drinking water. Subsequently, a 24 hour urine was collected. After completion of the collection period blood was obtained and the animals were sacrificed to determine tissue Al concentrations of trabecular bone, liver, spleen, brain, and muscle on day 42. An identical protocol was carried out, separately, in rats with normal renal function.

In situ *intestinal perfusion*

To assess the effect of Fe status on the intestinal absorption of Al directly at the organ level perfusions of *in situ* rat gut preparations were performed. Rats with normal renal function and either Fe overload or Fe deficiency and pair-fed controls with normal Fe status were fasted for 48 hours with free access to drinking water. Following anesthesia with 45 mg/kg pentobarbital the abdomen was opened by a midline incision and an afferent silicone catheter (internal diameter 0.5 mm) was inserted into the proximal jejunum 1 cm past the ligament of Treitz. The efferent limb was a polyvinyl chloride cannula (length 8 cm, internal diameter 3 mm) inserted proximal to the ileocecal juncture at the insertion of the ileocecal ligament. A small intestinal segment of approximately 100 cm length was perfused. Prior to perfusion the intestine was returned to the peritoneal cavity and the abdomen was closed with sutures. In experiments comparing uremic rats with pair-fed controls ureters were ligated to prevent elimination of absorbed Al. Perfusion experiments were performed in an air temperature-controlled hood and the animals' body temperature was kept at 37°C. During a 30-minute equilibration period the intestinal segment was perfused at a rate of 1 ml/min with an Al-free solution containing 138 mM NaCl, 4.5 mM KCl, 1.25 $CaCl_2$, 2.0 mM glucose, and 2.5 mM sodium lactate adjusted to pH 4.0. Subsequently, Al absorption was studied during a 75-minute absorption period using an equimolar perfusate solution containing 2.5 mM Al lactate. To assess water fluxes and permeability of the paracellular intestinal pathway 200 mg/liter ^{3}H-inulin (specific radioactivity 0.075 mCi/g) and 1 mM ^{14}C-mannitol (specific radioactivity 1.5 mCi/mol) were added to the solution in some experiments during the equilibration period as well as during the absorption period. To block the paracellular pathway additional perfusions were carried out using a solution containing 2 mM kinetin. All perfusate solutions were freshly prepared and were kept at 37°C during the perfusion experiments. A totally occlusive roller pump (Desaga, Heidelberg, Germany) was used to pump the perfusate to the afferent catheter. Perfusion pressure was continuously monitored and was kept well below 30 cm water. Effluent perfusate was drained by gravity into polypropylene collection tubes in a fraction collector (Redi Frac, Pharmacia LKB, Uppsala, Sweden) in five minute intervals. Blood samples of 200 μl were drawn via a cannula inserted into the right jugular vein at the start of perfusion with Al-containing media and after 15, 30, 45, 60, and 75 minutes of the absorption period. In addition, blood of the portal vein was collected at completion of the absorption period (75 min).

Analytical procedures

Aluminum in serum and urine was determined by flameless atomic absorption spectrophotometry using a 2380 spectrophotometer equipped with a HGA-400 flameless furnace and an AS-40 automatic sample injector (Perkin-Elmer) by a modification [17] of a previously described method [15]. For Al determination in bone, tibiae were bisected and marrow was removed with a jet of deionized water. Dried samples of cancellous bone were ground to powder in an agate mill, defatted (petroleum ether/ether, 1:1 vol:vol), digested with nitric acid (Suprapur; Merck, Darmstadt, Germany; Al content < 148 nM), diluted with distilled water and analyzed as reported earlier [37, 38]. Similarly, for Al determination in liver, spleen, brain, muscle, and duodenal mucosa dried aliqotes were weighed, ground and analyzed following digest with nitric acid. Muscle samples were defatted, redried and reweighed prior to the digest [39].

Creatinine in serum was measured by an autoanalyzer (Synchron Astra 4; Beckman, Fullerton, CA, USA). Red blood cell count, hematocrit, hemoglobin, and erythrocyte indices were measured by a multichannel autoanalyzer (Autolyzer 801; Contraves, Zurich, Switzerland).

For nonheme Fe determination tissues were dried (120°C, 16 hr) and ground to powder in an agate mill. Aliquotes (20 mg) were digested with 150 μl of a 1:1 (vol:vol) mixture of concentrated sulphuric and nitric acids by heating for 15 minutes over a low flame [40]. After cooling to ambient temperature the colorless residue was diluted with distilled water and its Fe content determined photometrically using bathophenanthroline-disulfonic acid as color reagent (Merckotest; Merck).

Paracellular permeability was assessed by quantitation of ^{14}C-mannitol absorption using ^{3}H-inulin as a non-absorbable marker. From each collected five-minute fraction of the intestinal effluent 1 ml aliquotes were counted in a scintillation fluid (Hionic Fluor; Packard, Downers Gove, IL, USA) using a 1215 Rackbeta II (LKB, Uppsala, Sweden) scintillation counter. The absorption of mannitol relative to inulin was calculated by standard equations and was corrected for the length of the perfused intestinal segment [41].

Aluminum lactate was purchased from Riedel-de Haën (Seelze, Germany) and sodium lactate was supplied by Fluka (Buchs, Switzerland). Kinetin was purchased from Serva (Heidelberg, Germany), ^{3}H-inulin and ^{14}C-mannitol were supplied by Amersham Buchler (Braunschweig, Germany). All other chemicals were obtained from Merck.

Reported data are expressed as mean value ± 1 SD except where noted otherwise. Statistical probabilities are derived from the Kruskal-Wallis test, a non-parametric analysis of variance, followed by a sequentially rejective multiple test procedure to compare specific groups [42]. A value of $P < 0.05$ was considered significant. Correlation between two parameters was accomplished by Spearman rank correlation.

Table 1. Hematologic examinations, serum creatinine and iron (Fe) concentration in liver and duodenal mucosa in iron-depleted rats (Fe−) and iron-overloaded rats (Fe+) with either normal renal function (C) or chronic renal failure (Nx) and in appropriate controls (N = 25 per group)

Group	Hemoglobin *g/liter*	Hematocrit *%*	Erythrocytes *Tera/liter*	Creatinine *μmol/liter*	Liver Fe *μmol/g*	Duodenal Fe *μmol/g*
C	13.8 ± 1.2	45.1 ± 4.3	8.5 ± 0.9	43 ± 6	7.14 ± 1.61	1.59 ± 0.22
C+Fe−	6.4 ± 0.9[a,b]	27.4 ± 2.6[a]	6.3 ± 0.6[a]	45 ± 4	1.34 ± 0.16[a,b]	0.68 ± 0.11[a,b]
C+Fe+	13.7 ± 0.6	45.0 ± 1.3	8.3 ± 0.3	41 ± 6	55.69 ± 13.20[a]	3.17 ± 0.82[a]
Nx	8.3 ± 2.2	37.6 ± 10.3	6.0 ± 1.6	124 ± 43[c]	10.03 ± 1.74	1.30 ± 0.27
Nx+Fe−	5.9 ± 1.2[c,d]	29.4 ± 5.0[c,d]	5.1 ± 0.6[d]	110 ± 15[c]	1.30 ± 0.25[c,d]	0.59 ± 0.25[c,d]
Nx+Fe+	12.5 ± 0.5[c]	57.0 ± 2.4[c]	8.5 ± 0.5[c]	112 ± 13[c]	90.85 ± 13.77[c]	4.66 ± 0.98[c]

[a] $P < 0.05$ vs. C; [b] $P < 0.05$ vs. C+Fe+; [c] $P < 0.05$ vs. Nx; [d] $P < 0.01$ vs. Nx+Fe+; [e] $P < 0.001$ vs. all C groups.

Table 2. Urinary excretion of aluminum (Al) in iron-depleted rats (Fe−) and iron-overloaded rats (Fe+) with either normal renal function (C) or chronic renal failure (Nx) and in appropriate controls following an oral load of 407 μmol aluminum

Group	N	Urine Al *nmol/day* Pre-load	Post-load day 1	Post-load total excretion (5 days)
C	10	5 ± 3	141 ± 43	316 ± 65
C+Fe−	9	4 ± 1	82 ± 27	229 ± 85
C+Fe+	10	7 ± 4	102 ± 38	240 ± 59
Nx	7	17 ± 6	232 ± 152	500 ± 185[a–c]
Nx+Fe−	8	9 ± 2	267 ± 135	533 ± 234[a–c]
Nx+Fe+	9	8 ± 4	273 ± 156	536 ± 201[a–c]

[a] $P < 0.01$ vs. C; [b] $P < 0.05$ vs. C+Fe−; [c] $P < 0.01$ vs. C+Fe+

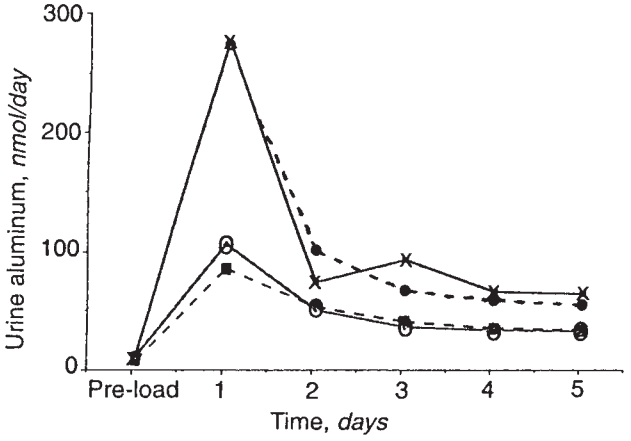

Fig. 1. *Time course of the urinary excretion of aluminum following a single oral dose of 407 μmol aluminum in iron-overloaded uremic rats (Nx+Fe+, —×—) and in iron-depleted uremic rats (Nx+Fe−;--●--) and in respective controls (C+Fe+, —○—C+Fe− --■--) (error bars omitted).*

Results

Iron overloading and Fe depletion resulted in significant hematologic changes both in rats with normal renal function and in uremic rats (Table 1). Fe-depleted animals had significantly lower hemoglobin concentrations, a lower erythrocyte count and lower hematocrits as compared to their controls. In addition, changes in body Fe stores were confirmed by significant increases or decreases in liver Fe concentration as well as in the nonheme Fe concentration of duodenal mucosa. Baseline urinary excretion rates of Al were not affected by Fe deficiency in comparison to rats with normal Fe status or Fe-overloaded animals (Table 2). Following a single oral dose of Al urinary Al excretion rates increased several-fold in all study groups within the first post-load day (Fig. 1). Changes in Fe status had no effect on the magnitude of this increase regardless of renal function. However, independent of changes in Fe status Al excretion tended to be greater in uremics as compared to their respective controls. Therefore, post-load total urinary excretion of Al over five days was significantly larger in uremic rats versus control rats regardless of the Fe status, and vice versa the Fe status had no effect on the post-load total excretion of Al in either renal function group.

In analogy, measurement of serum Al concentrations showed a similar pattern of post-load increases in all groups (Table 3). Baseline fasting serum concentrations of Al were comparable between all groups. One hour after ingestion of Al there was a significant increase in serum Al concentrations, the magnitude of which was again not affected by alterations in Fe status. In contrast, irrespective of Fe status uremic rats had significantly higher post-load one-hour serum Al concentrations as compared to their respective controls. Five hours after the oral Al load serum Al was elevated in each group to a similar degree regardless of renal function or Fe status. Twenty-four hour post-load serum Al tended to drop back to baseline values, but was still significantly elevated in group Nx+Fe+. Prior to the oral load Al concentrations in duodenal mucosa were higher in Nx rats as compared to controls (Table 4). After five hours post-load mucosal Al was significantly elevated in all treatment groups. Fe

Table 3. Pre-load and post-load serum concentrations of aluminum (Al) in iron-depleted rats (Fe−) and iron-overloaded rats (Fe+) with either normal renal function (C) or chronic renal failure (Nx) and in appropriate controls loaded with a single oral dose of 407 μmol aluminum

Group	N	Serum Al concentration *nM* Pre-load	Post-load 1 hour	Post-load 6 hours	Post-load 24 hours
C	10	43 ± 11	207 ± 54[a]	661 ± 170[a,b]	115 ± 70
C+Fe−	10	40 ± 4	204 ± 130[a]	530 ± 152[a,b]	122 ± 89
C+Fe+	10	45 ± 12	248 ± 63[a]	481 ± 126[a,b]	94 ± 81
Nx	10	41 ± 11	524 ± 271[a,c,d,e]	667 ± 159[a]	156 ± 104
Nx+Fe−	10	41 ± 12	433 ± 204[a,c,d,e]	485 ± 181[a]	139 ± 96
Nx+Fe+	10	42 ± 12	374 ± 115[a,c,d]	556 ± 126[a,b]	133 ± 48[a]

[a] $P < 0.05$ vs. pre-load; [b] $P < 0.05$ vs. 1 hr post-load; [c] $P < 0.05$ vs. C; [d] $P < 0.05$ vs. C+Fe−; [e] $P < 0.05$ vs. C+Fe+

Table 4. Aluminum (Al) concentrations in duodenal mucosa subsequent to an oral load of 407 μmol aluminum in iron-depleted rats (Fe−) and iron-overloaded rats (Fe+) with either normal renal function (C) or chronic renal failure (Nx) and in appropriate controls

		Mucosal Al *nmol/g*		
Group	*N*	Pre-load	Post-load 5 hours	Post-load 24 hours
C	8	9 ± 9	89 ± 28[a,b]	48 ± 17
C+Fe−	8	8 ± 9	109 ± 18[a,b]	37 ± 16
C+Fe+	8	6 ± 5	108 ± 47[a,b]	56 ± 19
Nx	8	35 ± 9[c−e]	81 ± 31[a]	92 ± 34[c−g]
Nx+Fe−	8	29 ± 8[c−e]	94 ± 37[a]	62 ± 39[d]
Nx+Fe+	8	33 ± 8[c−e]	79 ± 38[a,b]	131 ± 21[c−f]

[a] $P < 0.05$ vs. pre-load; [b] $P < 0.05$ vs. post-load 24 hr; [c] $P < 0.01$ vs. C; [d] $P < 0.05$ vs. C+Fe−; [e] $P < 0.01$ vs. C+Fe+; [f] $P < 0.05$ vs. Nx+Fe−; [g] $P < 0.01$ vs. Nx+Fe+

Table 5. Urinary excretion of aluminum (Al) subsequent to an intravenous load of 370 nmol Al in iron-depleted rats (Fe−) and iron-overloaded rats (Fe+) with either normal renal function (C) or chronic renal failure (Nx) and in appropriate controls

		Urine Al *nmol/day*		
Group	*N*	Pre-load	Post-load day 1	Post-load total excretion (5 days)
C	10	10 ± 2	210 ± 37	287 ± 47
C+Fe−	9	7 ± 1	186 ± 81	249 ± 77
C+Fe+	10	10 ± 2	168 ± 16	250 ± 15
Nx	7	14 ± 7	170 ± 57	267 ± 67
Nx+Fe−	8	15 ± 5	116 ± 42	209 ± 39[a]
Nx+Fe+	9	13 ± 3	76 ± 30	144 ± 34[a,b]

[a] $P < 0.01$ vs. C; [b] $P < 0.05$ vs. C+Fe−, C+Fe+, Nx, Nx+Fe−

deficiency did not increase mucosal Al nor did Fe overload reduce mucosal Al accumulation. Twenty-four hours after the load mucosal Al was still significantly elevated in uremic rats and this tissue burden of Al was most marked in Fe-overloaded animals. In contrast, rats with normal renal function had lower mucosal Al, however, again Fe-overloaded rats tended to have the highest tissue burden of Al. Following an intravenous load of Al urinary excretion of Al was similar among rats with normal renal function (Table 5). Therefore, Fe status did not affect the total Al recovery in urine in the control groups. In uremic rats, however, post-load total excretion was significantly lower in group Nx+Fe+ versus all other groups. When Al was administered orally together with Fe chloride to rats with normal renal function and with normal body Fe stores, the post-load daily urinary Al excretion rates tended to be larger in this group as compared to a control group given Al together with sodium chloride (Table 6). Total post-load excretion after five days was significantly larger in the group given Fe and Al orally.

Long-term dietary exposure to aluminum

At the completion of the experimental period liver Fe and duodenal Fe had changed to a similar degree in Fe deficiency and Fe overload as compared to respective animals of the single dose studies (data not shown). As compared to pre-load values serum Al concentrations rose significantly in uremic rats and in controls

Table 6. Urinary excretion of aluminum (Al) in control rats (normal renal function, normal body iron stores) following an oral load of either 204 nmol Al ($AlCl_3$) with 204 nmol NaCl or 204 nmol Al together with 204 nmol iron (Fe) ($FeCl_3$]

		Urine Al *nmol/day*		
Group	*N*	Pre-load	Post-load day 1	Post-load total excretion (5 days)
Al+NaCl	8	4 ± 4	58 ± 38	87 ± 52
Al+Fe	8	5 ± 8	137 ± 71	170 ± 80[a]

[a] $P < 0.05$ vs. Al+NaCl

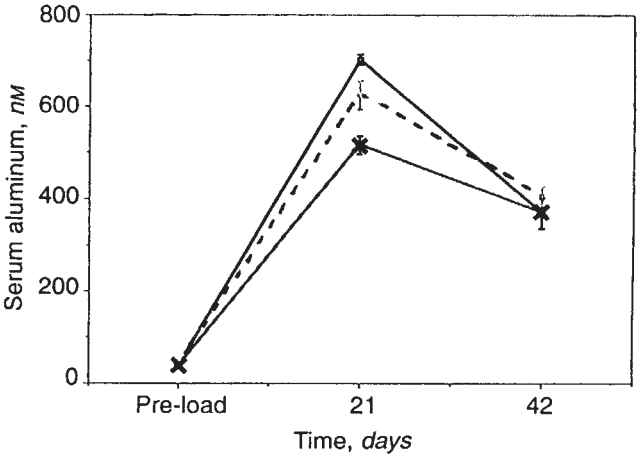

Fig. 2. *Serum aluminum (Al) concentrations in iron-depleted rats (C+Fe−, —×—) and iron-overloaded rats (C+Fe+, ---) with normal renal function (N = 10 per group) and in appropriate controls (C, ——) after exposure to Al-supplemented diet and Al-containing drinking water for 41 days (animals were fasted for 24 hr prior to serum Al on day 42) (mean ± SE).* Serum aluminum concentrations at days 21 and 42 were significantly different from respective baseline values ($P < 0.001$).

at day 21 (Figs. 2 and 3). This increase in serum Al was more marked in uremic rats, but was not affected by changes in body Fe stores. If anything, Fe-depleted rats tended to have slightly lower values. At day 42, 24 hours after removal of Al-containing feed and Al-containing drinking water serum Al concentrations had dropped by 47% and 44% in controls and in uremic rats, respectively. Again, neither this decline in serum Al nor absolute values were affected by Fe status. The corresponding urinary Al excretion rates revealed higher Al excretion in uremic rats as compared to controls (Fig. 4). Neither Fe overload nor Fe deficiency seemed to affect Al excretion subsequent to long-term dietary exposure. Cumulative intake of Al with the drinking water was similar among rats with a given renal function (C, 15.1 ± 4.8; C+Fe−, 14.6 ± 6.1; C+Fe+, 13.9 ± 5.6 mmol) but was higher in Nx-rats (Nx, 24.5 ± 9.0; Nx+Fe−, 26.1 ± 8.7; Nx+Fe+, 23.8 ± 5.2 mmol). On the contrary, total ingestion of food Al was lower in uremic rats as compared to controls, but was not different between the respective Fe-overloaded or Fe-deficient groups (Nx 181 ± 43 vs. C 304 ± 79 mmol, $P < 0.01$; Nx+Fe− 158 ± 61 vs. C+Fe− 334 ± 44 mmol, $P < 0.05$; Nx+Fe+ 167 ± 72 vs. C+Fe+ 328 ± 93 mmol, $P < 0.01$). Tissue Al concentrations tended to be elevated in rats with normal renal function as compared to

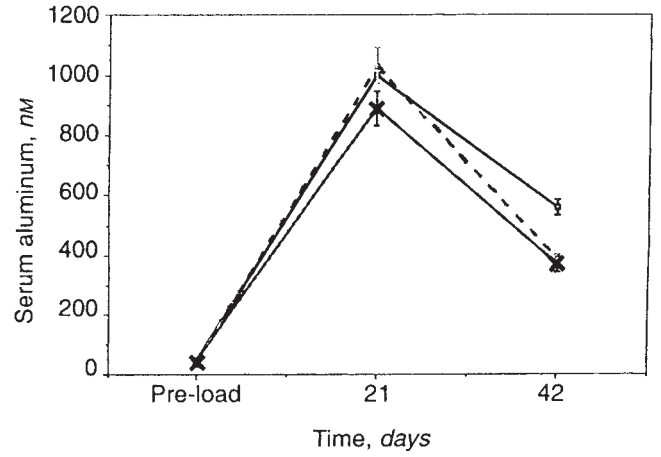

Fig. 3. *Serum aluminum (Al) concentrations in iron-depleted rats (Nx+Fe−, —×—) and iron-overloaded rats (Nx+Fe+, ---) with impaired renal function (N = 10 per group) and in appropriate controls (Nx, ——) after exposure to Al-supplemented diet and Al-containing drinking water for 41 days (animals were fasted for 24 hr prior to serum Al on day 42) (mean ± SE).* Serum aluminum concentrations at days 21 and 42 were significantly different from respective baseline values ($P < 0.001$).

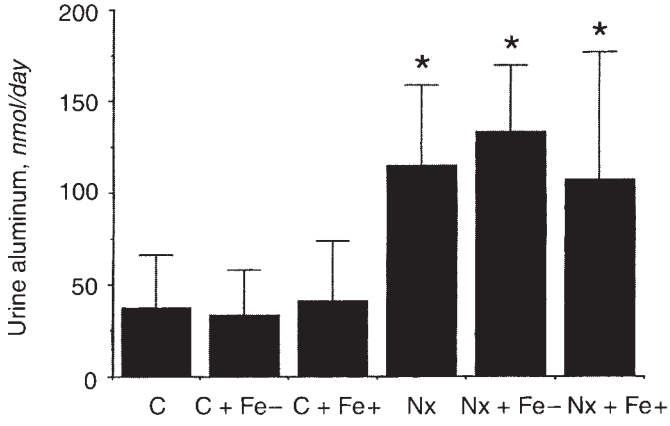

Fig. 4. *Urinary aluminum (Al) excretion rates in iron-depleted rats (Fe−) and iron-overloaded rats (Fe+) with either normal renal function (C) or chronic renal failure (Nx) and in appropriate controls (N = 10 per group) after exposure to Al-supplemented diet and Al-containing drinking water for 41 days (*P < 0.05 vs. C, C+Fe−, and C+Fe+).*

Table 7. Tissue aluminum (Al) concentrations (nmol/g) in iron (Fe)-depleted rats (C+Fe−) and iron-overloaded rats (C + Fe+) with normal renal function and in appropriate controls (C) after exposure to Al-supplemented diet and Al-containing drinking water for 41 days (*N* = 10 per group)

Group	Liver	Spleen	Muscle	Brain	Bone
Basline	159 ± 22	194 ± 20	48 ± 4	104 ± 37	219 ± 119
C	328 ± 121[a]	381 ± 219[a]	81 ± 11	130 ± 59	433 ± 130[a]
C+Fe−	276 ± 125[a]	308 ± 62[a]	85 ± 15	107 ± 15	433 ± 174[a]
C+Fe+	251 ± 71[a]	511 ± 129[a,b]	100 ± 33	104 ± 44	485 ± 141[a]

[a] $P < 0.05$ vs. bascline; [b] $P < 0.05$ vs. C+Fe−

Table 8. Tissue aluminum (Al) concentrations (nmol/g) in iron (Fe)-depleted rats (Nx+Fe−) and iron-overloaded rats (Nx+Fe+) with impaired renal function and in appropriate controls (Nx) after exposure to Al-supplemented diet and Al-containing drinking water for 41 days (*N* = 10 per group)

Group	Liver	Spleen	Muscle	Brain	Bone
Baseline	185 ± 70	216 ± 31	44 ± 4	96 ± 33	226 ± 100
Nx	330 ± 81[a]	407 ± 163[a]	67 ± 30	156 ± 63	556 ± 193[a]
Nx+Fe−	330 ± 119[a]	389 ± 119[a]	26 ± 7	156 ± 78	504 ± 185[a]
Nx+Fe+	437 ± 67[a]	514 ± 67[a,b]	52 ± 33	174 ± 100	548 ± 215[a]

[a] $P < 0.05$ vs. baseline; [b] $P < 0.05$ vs. Nx+Fe−

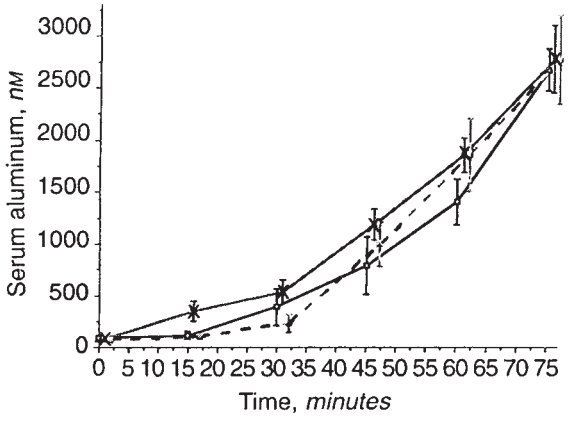

Fig. 5. *Serum aluminum (Al) concentrations in iron-depleted rats (C+Fe−, —×—) and iron-overloaded rats (C+Fe+, --○--) with normal renal function (N = 6 per group) and in appropriate controls (C,—) during in situ small intestinal perfusion with an Al-containing perfusion solution over a period of 75 minutes [mean ± standard error].* Serum aluminum concentrations at minutes 30 to 75 were significantly different from respective baseline values ($P < 0.01$).

baseline values. However, this increase attained significance only in liver, spleen and bone (Table 7). The increase in tissue Al concentrations followed a similar pattern in uremic groups (Table 8). Again, significant increments were observed in bone, liver, and spleen. Irrespective of renal function, Fe deficiency did not seem to increase the tissue Al burden as compared to Fe-overloaded groups or to controls with normal Fe status. In particular, there was no pronounced elevation of brain Al in Fe-deficient rats. The increase in spleen Al was even significantly larger in Fe-overloaded rats as compared to group C+Fe−. Similarly, spleen Al was highest in group Nx+Fe+ and was significantly different from the post-load spleen Al in group Nx+Fe−.

In situ *intestinal perfusion studies*

When rats with intact kidneys were perfused with an Al-containing perfusate, a continuous time-dependent increase in serum Al concentrations was observed (Fig. 5). After 30 minutes of perfusion there was a parallel increase in serum Al concentrations in all three groups. Systemic peak serum Al concentrations after 75 minutes of exposure did not differ among the three experimental groups and compared well with the respective Al concentrations in portal vein blood (C, 2685 ± 489 vs. 4133 ± 948 nM, $P < 0.01$; C+Fe−, 2770 ± 756 vs. 4555 ± 1427 nM, P = NS; C+Fe+, 2763 ± 933 vs. 4067 ± 837 nM, $P < 0.05$). When paracellular permeability was assessed, control rats absorbed very little mannitol from the perfused intestinal segment relative to inulin throughout the 75 minutes of perfusion with the standard

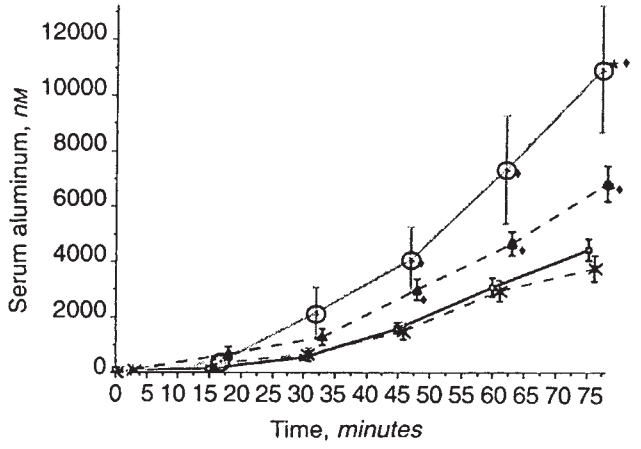

Fig. 6. *Serum aluminum (Al) concentrations in uremic rats (Nx) during in situ small intestinal perfusion with an Al-containing perfusion solution with or without kinetin added and in appropriate controls (C) with normal renal function (N = 6 per group) (mean ± SE).* *$P < 0.01$ versus Nx, ♦ denotes $P < 0.05$ versus respective controls. Serum aluminum concentrations at minutes 30 to 75 were significantly different from respective baseline values ($P < 0.01$). Symbols are: —■—, C; --▲--, Nx + kinetin; —○—, Nx; --×--, C+ kinetin.

solution (16.7 ± 20.5 μl/hr/cm, $N = 7$), and this finding was not affected by differences in Fe status. In contrast, mannitol absorption was significantly higher in uremic animals as compared to the controls (64.5 ± 34.5 μl/hr/cm, $P < 0.01$, $N = 7$). In a subsequent experiment, control rats with unaltered Fe status and pair-fed uremic animals were perfused with either standard solution or a perfusion solution containing kinetin (Fig. 6). In rats with intact kidneys the addition of kinetin had no marked affect on the time-dependent increase in serum Al concentrations. At variance, uremic rats perfused with kinetin showed a divergent pattern. Kinetin caused a progressively smaller increase in serum Al concentrations in comparison to uremic rats treated under standard perfusion conditions. Although the increase in serum Al was lower in the Nx+kinetin group, the slope of the time-dependent increase in serum Al was still steeper than the slope in control rats, and serum Al concentrations were significantly higher after 45, 60, and 75 minutes.

Discussion

Aluminum and iron share several physicochemical properties, and recent studies have shown that intense interrelationships exist between the metabolism of both elements. In serum the predominant fraction of aluminum is non-ultrafiltrable and largely bound to transferrin [22, 23]. There is ample experimental evidence that transferrin mediates not only serum transport but also cellular uptake and toxicity of aluminum. Therefore, cellular uptake of transferrin-bound aluminum via transferrin receptors on cell surfaces and via subsequent internalization by receptor-mediated endocytosis has been shown in a variety of cell lines, namely neuroblastoma cells, erythroleukemia cells, hematopoietic progenitor cells, osteosarcoma cells, hepatocytes, and lymphocytes [24, 43–47]. Intracellularly, aluminum may interfere with iron metabolism at several, less well defined sites including ferritin regulation by iron, utilization of iron for the synthesis of hem, and expression of the transferrin receptor [21, 48].

In addition, it is now also widely believed that aluminum absorption occurs via iron-specific, transferrin-dependent pathways and that iron deficiency enhances the fractional absorption of aluminum [49]. This assumption is deduced from several observations. (a) Clinical studies reported a negative relationship between serum ferritin and serum aluminum concentrations in dialysis patients [28–30]. (b) One uncontrolled study found elevated urinary excretion rates of aluminum in renal failure patients with low serum ferritin, suggesting increased intestinal absorption [33]. (c) As it was the case in other cell lines, the rat intestinal cell line RIE1 takes up aluminum via the transferrin receptor and iron deficiency enhances this uptake [18, 24]. (d) A recent animal experiment claimed to provide evidence for a clear-cut relationship between iron deficiency and enhanced aluminum absorption [18]. However, each of these lines of evidence has its obvious shortcomings, and on the basis of the experimental data provided by the present study the effect of iron status on the intestinal absorption of aluminum needs a reappraisal. In particular, our study challenges many, if not all, of the previously published experimental data suggesting that iron-deficient rats reproducibly absorb more aluminum than do iron-replete controls [18]. As regards the clinical studies a negative correlation between the serum concentrations of ferritin and aluminum could also be explained by the fact that the degree of iron saturation may affect aluminum binding to transferrin. Support for this alternative explanation stems from experimental data of D'Haese et al, who demonstrated that a gradual increase in iron-transferrin saturation caused a progressive decrease not only in free binding sites available for aluminum, but also in the affinity of apotransferrin for aluminum [50]. In addition, since the increment in serum aluminum following a desferrioxamine test did not correlate with serum ferritin in the study of Huang et al, there was no evidence that the tissue burden of aluminum was more elevated in iron-deficient patients [28]. Moreover, other clinical investigations that addressed the effect of body iron stores on the intestinal absorption of aluminum more directly have yielded conflicting results [11]. While one study in dialysis patients demonstrated a negative correlation between serum iron concentration and the increment in serum aluminum after an oral aluminum load [30], another report indicated that simultaneous administration of iron supplementation together with aluminum-containing phosphate binders tended to enhance rather than to decrease aluminum absorption [31]. In the study that reported a larger urinary excretion of aluminum in renal failure patients with low serum ferritin the increase was small and differences in the dietary aluminum load were not assessed [33].

The data of the present study strongly argue against the hypothesis that iron deficiency causes hyperabsorption of aluminum. To make sure that our negative findings were correct, we carried out several independent series of experiments using different animal models comprising different doses of aluminum, variable durations of exposure to aluminum and various aluminum compounds. On purpose, we did not study citrate-containing diets despite the fact that citrate is an important determinant of the intestinal absorption of aluminum and that dietary constituents such as citrate have to be considered [11]. Citrate increases the intestinal absorption via the paracellular pathway, and this process is not likely to involve iron-specific carriers [14]. However,

besides citrate there are other complexing agents in the diet that increase the intestinal absorption of aluminum that may not affect the permeability of the paracellular pathway [16]. Our earlier studies demonstrated that lactate is one of them [17] and, consequently, we chose lactate rather than citrate in two of our experiments. Finally, a recent study that reported enhanced absorption of aluminum in iron-deficient rats used aluminum hydroxide and aluminum chloride but not citrate to dose the animals [18]. Each group of our three different experimental models had a well-defined body iron status. Measurement of nonheme iron concentrations in liver and in intestinal mucosa clearly confirmed marked alterations of body iron stores in the respective groups. However, neither single dose studies nor an extended exposure to dietary aluminum supported a role for iron status as an important modulator of aluminum absorption.

Following a single oral dose of aluminum chloride neither the post-load increment in serum aluminum nor urinary excretion rates of aluminum differed between iron-overloaded rats, iron-deficient animals and iron-replete controls with comparable renal function. The same findings applied to the increment in serum aluminum during *in situ* intestinal perfusion with aluminum lactate-containing perfusate. Again, the kinetics of serum aluminum were remarkably comparable between the three study groups. To exclude that the observed time course of post-load serum and urine aluminum was confounded by iron-related differences in compartmentalization or renal handling of aluminum, an intravenous aluminum dose was administered in separate experiments. Irrespective of iron status intravenous delivery of aluminum yielded similar cumulative excretion rates in control and treatment groups with intact kidneys, suggesting that interaction of iron and aluminum had not disturbed the interpretation of the urinary excretion rates of aluminum. In iron-overloaded uremic rats, however, urinary recovery of aluminum was significantly reduced as compared to group Nx+Fe−. If anything, measurement of urine aluminum may underestimate fractional absorption of aluminum in the presence of iron overload and uremia. This particular phenomenon may have led to the erroneous conclusion of hyperabsorption of aluminum in iron deficiency as compared to iron overload and may thus have contributed to contradictory data in the past [18, 32, 33].

The findings of our short-term single dose experiments apply also to a prolonged dietary exposure to aluminum over 41 days. As has been observed previously by other investigators [51], this maneuver resulted in an increase in bone aluminum as well as in aluminum concentrations in liver and spleen, indicating that these organs are the major sites of aluminum storage [1, 52]. Again, we could not provide any evidence that iron deficiency had increased aluminum absorption. The increase in bone aluminum was comparable among groups with similar renal function and spleen aluminum was even larger in iron-overloaded rats. Serum and urinary aluminum did not differ among groups with similar renal function and, what may be of particular interest, iron deficiency was not associated with excessive accumulation of aluminum in brain. Our data are in line with a previous investigation that showed no effect of iron deficiency on blood or liver aluminum concentrations in rats fed aluminum-rich tea leaves [32], and our findings are at odds with a report indicating that iron deficiency enhances aluminum absorption and deposition in brain [18]. However, the latter study did not report aluminum concentrations in the target organs bone, liver, and spleen where the major fraction of a systemic aluminum burden is deposited, and measurements of low levels of brain aluminum is subject to many analytical problems [1, 39].

Our findings contrast with recent evidence that iron deficiency enhances the cellular uptake of transferrin-bound aluminum by a rat intestinal epithelial cell line [24]. Therefore, it was suggested that transferrin should mediate the absorption of aluminum and that increased expression of intestinal transferrin receptors should lead to increased aluminum absorption in iron deficiency [24, 49]. However, the relevance of transferrin receptor-mediated uptake of aluminum for the intestinal absorption process is questionable. In intestinal epithelial cells transferrin receptors are not expressed at the apical surface and are thus not available to intraluminal aluminum [25, 26, 53, 54]. Consequently, recent studies have shown that transferrin is unlikely to be the mucosal regulator of iron absorption [53, 54, 55]. Therefore, individuals with atransferrinemia have increased rather than decreased total body iron stores [56], and the gene for transferrin is not expressed in duodenal mucosal cells [57, 58]. Instead, it appears that iron uptake from the gut is facilitated by a transferrin-independent transport system utilizing $\beta 3$ integrin and mobilferrin, a homologue of calreticulin [55, 59–61]. Although these proteins may also bind zinc, cobalt, and lead, their interaction with aluminum remains speculative and needs to be investigated [53]. In the present study peroral co-administration of aluminum and iron did not reduce, but tended to enhance aluminum absorption. This seems to argue against competitive binding to, and apical aluminum uptake by these iron-specific carriers. In addition, when we measured mucosal aluminum concentrations, there was an increase five hours post-load that persisted in uremic animals, but this increment was not augmented by iron deficiency. If anything, mucosal aluminum concentrations tended to be higher in iron-overloaded uremic rats after 24 hours. Our data are in line with recent findings demonstrating that in mucosal cytosol extracts aluminum may associate with two proteins, neither of which bind iron [62].

The present study confirms that the intestinal absorption of aluminum is enhanced in the uremic state. This has been a consistent phenomenon in a series of previous experiments, however, the cause of this impaired barrier function of the intestine has not been fully elucidated [11, 15, 17, 63, 64]. The magnitude of aluminum absorption in the remnant kidney rat model is positively correlated with severity and duration of renal failure, but enhanced absorption cannot be attributed to the degree of hyperparathyroidism, to calcitriol deficiency or to changes in gastric acid secretion [11]. This study clearly demonstrates that the increase in aluminum absorption in uremia is not related to subtle or overt iron deficiency since uremic rats had similar liver iron concentrations as compared to controls. Instead, our data are compatible with the notion that the increase in the fractional absorption of aluminum may be related to enhanced paracellular permeability of the intestine. Evaluation of intestinal permeability in rats by other investigators has indicated reduced permeability in acute renal failure and increased permeability in chronic renal failure [65, 66]. Morphological and functional alterations of the small intestine in uremia are well established [67–69], and these changes may be involved in the pathogenesis of disturbed paracellular permeability. We noted increased intestinal mannitol absorption in uremic rats suggesting a substantial paracellular shunt pathway irrespective of iron status. Blockade of

paracellular permeability by means of kinetin resulted in a blunted increase in serum aluminum concentrations during intestinal perfusion with aluminum lactate consistent with the view that excess absorption in uremia had occurred via the transepithelial, extracellular pathway. Since kinetin did not completely normalize the time course of serum aluminum as compared to controls, additional, noniron-dependent transcellular mechanisms may be operating as well to facilitate aluminum absorption in renal failure. It is noteworthy that kinetin did not reduce the time-dependent increment in serum aluminum in controls. Given the low intestinal permeability in the presence of normal renal function, this would imply that the usual site of aluminum absorption is via the transcellular pathway. Exceptional transepithelial movement of aluminum along the paracellular pathway has only been demonstrated following administration of citrate due to chelation of calcium and subsequent opening of cellular tight junctions [14]. Apparently, uremic enteropathy may constitute another condition where epithelial leackage allows paracellular aluminum permeation across the intestinal barrier.

In conclusion, the present study provides several lines of evidence against the previously held view that intestinal absorption of aluminum occurs via iron-specific or via transferrin-dependent pathways. This negative finding may be related to the neglected fact that transferrin receptors are not expressed on the absorptive surface of intestinal epithelial cells and that absorption of iron is instead facilitated by the mucin-mobilferrin-integrin pathway. In addition, changes in body iron status do not affect the enhanced aluminum absorption in the uremic state. This phenomenon is in part due to increased paracellular permeability. Further clinical and experimental studies using the isotope ^{26}Al in conjunction with accelerator mass spectrometry are needed to confirm these observations [70].

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